
BIOCHEMISTRY

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To:

*Our parents, who encouraged us,
Our teachers, who enabled us, and
Our children, who put up with us.*

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Cover Art: One of a series of color studies of horse heart cytochrome c designed to show the influence of amino acid side chains on the protein's three-dimensional folding pattern. We have selected this study to symbolize the discipline of biochemistry: Both are beautiful but still in process and hence have numerous "rough edges." Drawing by Irving Geis in collaboration with Richard E. Dickerson.

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with its particular arrangement of four methyl, two propionate, and two vinyl substituents, is known as **protoporphyrin IX**. Heme, then, is protoporphyrin IX with a centrally bound iron atom. In Hb and Mb, the iron atom normally remains in the Fe(II) (ferrous) oxidation state whether or not the heme is oxygenated (binds O₂).

The Fe atom in deoxygenated Hb and Mb is 5-coordinated by a square pyramid of N atoms: four from the porphyrin and one from a His side chain of the protein. Upon oxygenation, the O₂ binds to the Fe(II) on the opposite side of the porphyrin ring from the His ligand so that the Fe(II) is octahedrally coordinated; that is, the ligands occupy the six corners of an octahedron centered on the Fe atom (Fig. 9-1). *Oxygenation changes the electronic state of the Fe(II)-heme as is indicated by the color change of blood from the dark purplish hue characteristic of venous blood to the brilliant scarlet color of arterial blood and blood from a cut finger (Fig. 9-2).*

Certain small molecules, such as CO, NO, and H₂S, coordinate to the sixth liganding position of the Fe(II) in Hb and Mb with much greater affinity than does O₂. This, together with their similar binding to the hemes of cytochromes, accounts for the highly toxic properties of these substances.

The Fe(II) of Hb or Mb can be oxidized to Fe(III) to form **methemoglobin (metHb)** or **metmyoglobin (metMb)**. MetHb does not bind O₂; its Fe(III) is already octahedrally coordinated with an H₂O molecule in the sixth liganding position. The brown color of dried blood and old meat is that of metHb and metMb. Erythrocytes (red blood cells) contain the enzyme **methemoglobin reductase**, which converts the small amount of metHb that spontaneously forms back to the Fe(II) form.

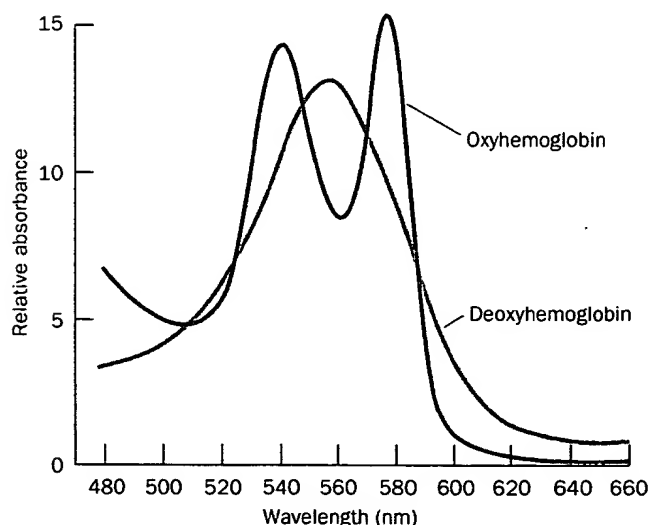
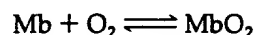


Figure 9-2
The visible absorption spectra of oxygenated and deoxygenated hemoglobins.

B. Oxygen Binding

The binding of O₂ to myoglobin is described by a simple equilibrium reaction



with dissociation constant

$$K = \frac{[\text{Mb}][\text{O}_2]}{[\text{MbO}_2]} \quad [9.1]$$

(biochemists usually express equilibria in terms of dissociation constants, the reciprocals of the more chemically traditional association constants). The O₂ dissociation of Mb may be characterized by its **fractional saturation**, Y_{O_2} , defined as the fraction of O₂-binding sites occupied by O₂.

$$Y_{\text{O}_2} = \frac{[\text{MbO}_2]}{[\text{Mb}] + [\text{MbO}_2]} = \frac{[\text{O}_2]}{K + [\text{O}_2]} \quad [9.2]$$

Since O₂ is a gas, its concentration is conveniently expressed by its partial pressure, p_{O_2} (also called the **oxygen tension**). Equation [9.2] may therefore be expressed:

$$Y_{\text{O}_2} = \frac{p_{\text{O}_2}}{K + p_{\text{O}_2}} \quad [9.3]$$

Now define p_{50} as the value of p_{O_2} when $Y_{\text{O}_2} = 0.50$, that is, when one half of myoglobin's O₂-binding sites are occupied. Equation [9.3] then indicates that $K = p_{50}$ so that our expression for the fractional saturation of Mb finally becomes:

$$Y_{\text{O}_2} = \frac{p_{\text{O}_2}}{p_{50} + p_{\text{O}_2}} \quad [9.4]$$

Hemoglobin Cooperatively Binds O₂

Myoglobin's O₂-dissociation curve (Fig. 9-3) closely follows the hyperbolic curve described by Eq. [9.4]; its p_{50} is 2.8 torr (1 torr = 1 mmHg at 0°C = 0.133 kPa; 760 torr = 1 atm). Mb therefore gives up little of its bound O₂ over a relatively wide range of p_{O_2} , for example, $Y_{\text{O}_2} = 0.97$ at $p_{\text{O}_2} = 100$ torr and 0.88 at 20 torr. In contrast, hemoglobin's O₂-dissociation curve (Fig. 9-3), which has a **sigmoidal** shape (S shape) that Eq. [9.4] cannot describe, indicates the amount of O₂ bound by Hb changes significantly over a relatively small range of p_{O_2} , for example, $Y_{\text{O}_2} = 0.95$ at 100 torr and 0.30 at 20 torr in whole blood. Hemoglobin's sigmoidal O₂-dissociation curve is of great physiological importance; it permits the blood to deliver much more O₂ to the tissues than it could if Hb had a hyperbolic O₂-dissociation curve resembling that of Mb. A sigmoidal dissociation curve is diagnostic of a **cooperative interaction** between a protein's small molecule binding sites; that is, the binding of one small molecule affects the binding of others. In this case, the binding of O₂ increases the affinity of Hb

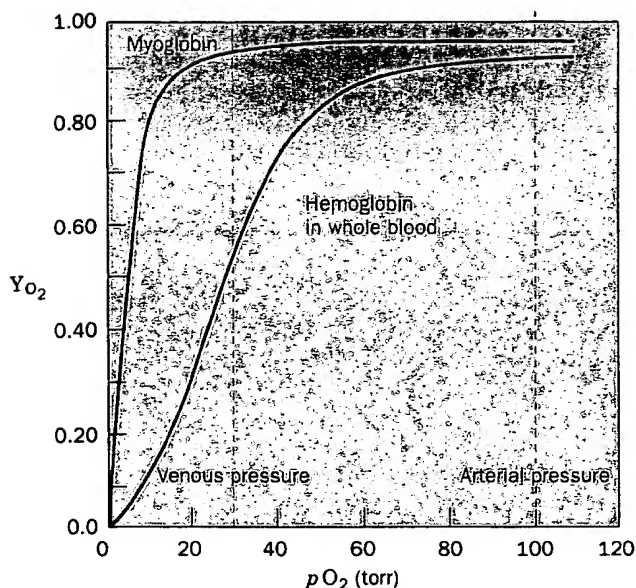


Figure 9-3

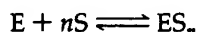
The oxygen dissociation curves of Mb and Hb in whole blood. The normal sea level values of human arterial and venous pO_2 values are indicated.

for binding additional O_2 . The structural mechanism of hemoglobin cooperativity is described in Section 9-2C.

The Hill Equation Phenomenologically Describes Hemoglobin's O_2 -Binding Curve

The earliest attempt to analyze hemoglobin's sigmoidal O_2 -dissociation curve was formulated by Archibald Hill in 1910. We shall follow his analysis in general form because it is useful for characterizing the cooperative behavior of oligomeric enzymes as well as that of hemoglobin.

Consider a protein E consisting of n subunits that can each bind a molecule S, which, in analogy with the substituents of metal ion complexes, is known as a **ligand**. Assume that the ligand binds with infinite cooperativity,



that is, the protein either has all or none of its ligand-binding sites occupied so that there are no observable intermediates ES_1 , ES_2 , etc. The dissociation constant for this reaction is

$$K = \frac{[E][S]^n}{[ES_n]} \quad [9.5]$$

and, as before, its fractional saturation is expressed:

$$Y_s = \frac{n[ES_n]}{n([E] + [ES_n])} \quad [9.6]$$

Combining Eqs. [9.5] and [9.6] yields

$$Y_s = \frac{n[E][S]^n/K}{n([E] + [S]^n/K)}$$

that upon algebraic rearrangement and cancellation of terms becomes the **Hill equation**:

$$Y_s = \frac{[S]^n}{K + [S]^n} \quad [9.7]$$

which, in a manner analogous to Eq. [9.4], describes the degree of saturation of a multisubunit protein as a function of ligand concentration.

Infinite ligand-binding cooperativity, as assumed in deriving the Hill equation, is a physical impossibility. Nevertheless, n may be taken to be a nonintegral parameter related to the degree of cooperativity among interacting ligand-binding sites rather than the number of subunits per protein. The Hill equation then becomes a useful empirical curve-fitting relationship rather than an indicator of a particular model of ligand binding. The quantity n , the **Hill constant**, increases with the degree of cooperativity of a reaction and thereby provides a convenient although simplistic characterization of a ligand-binding reaction. If $n = 1$, Eq. [9.7] describes a hyperbola as do Eqs. [9.3] and [9.4] for Mb, and the ligand-binding reaction is said to be **noncooperative**. A reaction with $n > 1$ is described as **positively cooperative**: Ligand binding increases the affinity of E for further ligand binding (cooperativity is infinite in the limit that n is equal to the number of ligand-binding sites in E). Conversely, if $n < 1$, the reaction is termed **negatively cooperative**: Ligand binding reduces the affinity of E for subsequent ligand binding.

Hill Equation Parameters May Be Graphically Evaluated

The Hill constant, n , and the dissociation constant, K , that best describe a saturation curve can be graphically determined by rearranging Eq. [9.7] as follows:

$$\frac{Y_s}{1 - Y_s} = \frac{[S]^n}{K + [S]^n} = \frac{[S]^n}{K}$$

and then taking the log of both sides to yield a linear equation:

$$\log \left(\frac{Y_s}{1 - Y_s} \right) = n \log [S] - \log K \quad [9.8]$$

The linear plot of $\log [Y_s/(1 - Y_s)]$ versus $\log [S]$, the **Hill plot**, has a slope of n and an intercept on the $\log [S]$ axis of $(\log K)/n$ (recall that the linear equation $y = mx + b$ describes a line with a slope of m and an x intercept of $-b/m$).

For Hb, if we substitute pO_2 for $[S]$ as was done for Mb, the Hill equation becomes:

$$Y_{O_2} = \frac{(pO_2)^n}{K + (pO_2)^n} \quad [9.9]$$

As in Eq. [9.4], let us define p_{50} as the value of pO_2 at

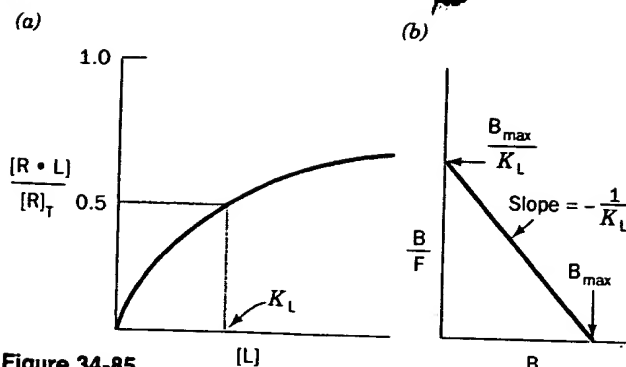
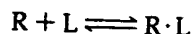


Figure 34-85
The binding of ligand to receptor: (a) A hyperbolic plot. (b) A Scatchard plot. Here, $B = [R \cdot L]$, $F = [L]$, and $B_{\max} = [R]_T$.

we have before. We begin, however, with a discussion of how receptor–ligand interactions are quantified.

Receptor Binding

Receptors, as do other proteins, bind their corresponding ligands (agonists and antagonists) according to the laws of mass action:



Here R and L represent receptor and ligand, and the reaction's dissociation constant is expressed:

$$K_L = \frac{[R][L]}{[R \cdot L]} = \frac{([R]_T - [R \cdot L])[L]}{[R \cdot L]} \quad [34.1]$$

where the total receptor concentration, $[R]_T = [R] + [R \cdot L]$. Equation [34.1] may be rearranged to a form analogous to the Michaelis–Menten equation of enzyme kinetics (Section 13-2A):

$$Y = \frac{[R \cdot L]}{[R]_T} = \frac{[L]}{K_L + [L]} \quad [34.2]$$

where Y is the fractional occupation of the ligand-binding sites. Equation [34.2] represents a hyperbolic curve (Fig. 34-85a) in which K_L may be operationally defined as the ligand concentration at which the receptor is half-maximally occupied by ligand.

Although K_L and $[R]_T$ may, in principle, be determined from an analysis of a hyperbolic plot such as Fig. 34-85a, the analysis of a linear form of the equation is a more accurate procedure. Equation [34.1] may be rearranged to:

$$\frac{[R \cdot L]}{[L]} = \frac{([R]_T - [R \cdot L])}{K_L} \quad [34.3]$$

Now, in keeping with customary receptor-binding nomenclature, let us redefine $[R \cdot L]$ as B (for bound ligand), $[L]$ as F (for free ligand), and $[R]_T$ as B_{\max} . Then Eq. [34.3] becomes:

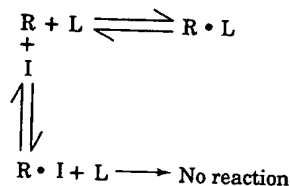
$$\frac{B}{F} = \frac{(B_{\max} - B)}{K_L} \quad [34.4]$$

A plot of B/F versus B , which is known as a Scatchard plot (after George Scatchard, its originator), therefore

yields a straight line of slope $-1/K_L$ whose intercept on the B axis is B_{\max} (Fig. 34-85b). Here, both B and F may be determined by filter-binding assays as follows. Most receptors are insoluble membrane-bound proteins and may therefore be separated from soluble free ligand by filtration (receptors that have been solubilized may be separated from free ligand by filtration, for example, through nitrocellulose; recall that proteins nonspecifically bind to nitrocellulose). Hence, through the use of radioactively labeled ligand, the values of B and F ($[R \cdot L]$ and $[L]$) may be determined, respectively, from the radioactivity on the filter and that remaining in solution. The rate of $R \cdot L$ dissociation is generally so slow (half-times of minutes to hours) as to cause insignificant errors when the filter is washed to remove residual free ligand.

Competitive-Binding Studies

Once the receptor-binding parameters for one ligand have been determined, the dissociation constant of other ligands for the same ligand-binding site may be determined through competitive-binding studies. The model describing this competitive binding is analogous to the competitive inhibition of a Michaelis–Menten enzyme (Section 13-3A):



where I is the competing ligand whose dissociation constant with the receptor is expressed:

$$K_I = \frac{[R][I]}{[R \cdot I]} \quad [34.5]$$

Thus, in direct analogy with the derivation of the equation describing competitive inhibition:

$$[R \cdot L] = \frac{[R]_T [L]}{K_L \left(1 + \frac{[I]}{K_I} \right) + [L]} \quad [34.6]$$

The relative affinities of a ligand and an inhibitor may therefore be determined by dividing Eq. [34.6] in the presence of inhibitor with that in the absence of inhibitor:

$$\frac{[R \cdot L]_i}{[R \cdot L]_0} = \frac{K_L + [L]}{K_L \left(1 + \frac{[I]}{K_I} \right) + [L]} \quad [34.7]$$

When this ratio is 0.5 (50% inhibition), the competitor concentration is referred to as $[I_{50}]$. Thus, solving Eq. [34.7] for K_I at 50% inhibition:

$$K_I = \frac{[I_{50}]}{1 + \frac{[L]}{K_L}} \quad [34.8]$$